ARTIFICIAL ALIEN POLLINATION AS A MODEL OF NATURAL INTROGRESSIVE HYBRIDIZATION ON THE EXAMPLE OF COTTON

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Besides the environmental factors inducing micro-or macro-mutations thus providing the variability, the most important role in the variability is played by cross pollination (anemophilia and entomophilia) in the result of which there is observed the introgressive hybridization.

At present time there are known numerous cases of introgressive hybridization between species and genera, though in practice it is impossible to obtain such hybrids artificially. The reason lies in the genetic and physiological incompatibility of the natural barrier of the crossed pairs.

We shall show several examples of introgressive hybridization and its significance in the evolution of plants. Introgressive hybridization includes four essential phases: (1) formation of hybrids, F_1; 2) spontaneous backcrossing, periodically repeated during a long period of time promoting the penetration of genetic material from one genus or species into the other through the incomplete isolation barrier; 3) outcrossing, to which even almost all the autogamcus plants are exposed in spite of the fact that potentially self- fertilization exists in all bisexual organisms; 4) natural selection of definite recombinants containing several new genes, selection which can give the beginning for their multiplication (in favourable conditions such forms can occupy a rather large area).

P. M. Zhukovskiy (1970) points out that in the result of spontaneous introgression there appeared many cultivated species of world significance: polyploid species of wheat, cotton, tobacco, rape and mustard, some species of *Rosa*, *Salix*, *Citrus* and many others. Many species originated by means of introgression on the diploid level: *Zea*, *Cerapadus*, Sorbocotone Aster and others. A striking example of the significance of spontaneous introgression in evolution is presented by modern maize, and the diversity of *Sorghum* genus united now into one [*Sorghum bicolor* (L) Moench.] species and the subfamily Aurantioideae, expecially genus *Citrus*. For the obtaining of artificial introgression it is recommended, for example, to create the joint populations with the participation of such species, as *Triticum timopheevi*, *T. militinae*, *Avena strigosa*, *A. sterilis*, *Beta palellaris*, *Gossypium nervosum*, *Cucurbita lundelliana* and many others. M. G. Popov (1928) wrote that ingrogression is the source of origin of new forms and races; it deforms old ones and plays an outstanding role in the evolution of the vegetative world. He referrs to intergeneric hybrid *Papaver* × *Rocmeria* as a kind of example or to 'Fergan plum' (*Amygdalus ulmifolia* × *Prunus cerasifera*).

It is widely known that tetraploid wheat appeared as the result of spontaneous crossing of wild and cultivated einkorn wheat (*T. thaoudar or T. boeoticum*) with diploid species *Aegilops speltoides* and the following spontaneous doubling of chromosomes. There exists a great diversity of new forms, races and species in *Lea* genus, in subfamily Aurantioideae, especially in *Citrus, Helianthus, Sorghum* and others, which appeared in analogous way. E. G. Bobrov (1944, 1961, 1972) comes to the conclusion that introgressive hybridization (in *Picea* and *Larix* genus) includes not only the nearest relative species, but many species belonging to different species, genera or to different sections of the genus. These data were supported by the palynological studies of D. B. Arkhangelskiy (1962). Apparently such tetraploid cottons as *G. barbadense* and *G. hirsutum*, tobacco, rustica tobacco, mustard and rape appeared spontaneously. Experimentally, in a similar way there were obtained, *Raphanobrassica* by Karpechenko and *Prunus domestica* by Ribina. Introgresive hybridization may give fertile distant hybrids to which belong: apple-tree-quince, wild *Cerapadus*, almond-peach and others. Analogous examples can be shown in a great number among arboreal, cereal and other crops. Thus, there are no doubts that introgressive hybridization along with mutations, polyploidy and other chromosome changes plays an extremely important role in the evolution of plants.

As further we are going to discuss the examples of introgressive hybridization in cotton it is necessary to give the description of spontaneous interspecific hybrids which were observed by many botanist and breeders. In this respect it is necessary to mention punctatum cottons (*G. hirsutum*) which travelled from their native land, eastern Mexico and Honduras, through the islands of Mexican Gulf to Bohe islands and penetrated into many countries of the Old World and forced out Asian cottons in Africa to the south from Sahara. Now they can be met on the coast of Eastern Africa and on the island of Indian Ocean and in southern India. During two hundred years they had greatly differentiated as the result of introgressive hybridization with *G. barbadense* and gave the beginning to "Indian weeds" (Hindi weeds) soiling the sowing of Upland and Egyptian varieties. Among these "weeds" there can be met forms with large bolls competing with Egyptian varieties according to the fibre quality; forms with high resistance to jassids. High resistance to bacterial wilt (*Xanthomonas malvacearum* Doton) is the result of introgression of African *punctatum* with Asian uplands. Apparently, the relative resistance of USA varieries to *Verticillium* wilt was obtained from *G. barbadense.*

After the successful voyage of Tur Heierdal on the papyrus ship "Ra-2" which proved the possibility of the contacts between American and African aborigenes, there were left no doubts concerning the introgressive hybridization between the *G. herbaceum* brought from Africa to America and spontaneous hybridization with *G. raimondii* or with some other diploid species and the further polyploidization or repeated natural backcrossing.

According to the report of Hutchinson (1959), Degener had discovered on one of the Hawaiian Isles the putative hybrid plant between *G. tomentosum* and

G. barbadense which usually do not cross. There can be given a number of such examples. Introgression, as a rule, takes place in such situation where there exists some genetic barrier between the parental types. The nearer the forms and races in their relationship, the less marked is the influence of the natural cross-pollination as the genotypes dissolved in the general gene pool.

Natural intercrossing is an extremely prolonged phenomenon and it demands decades of years, in case the repeated back pollination (back-crossing) doesn't take place. The experiments of Knight testify the positive significance of gene integration for the improving of plants by means of spontaneous interspecific hybridization with the participation of genes of some 'not-crossing' species.

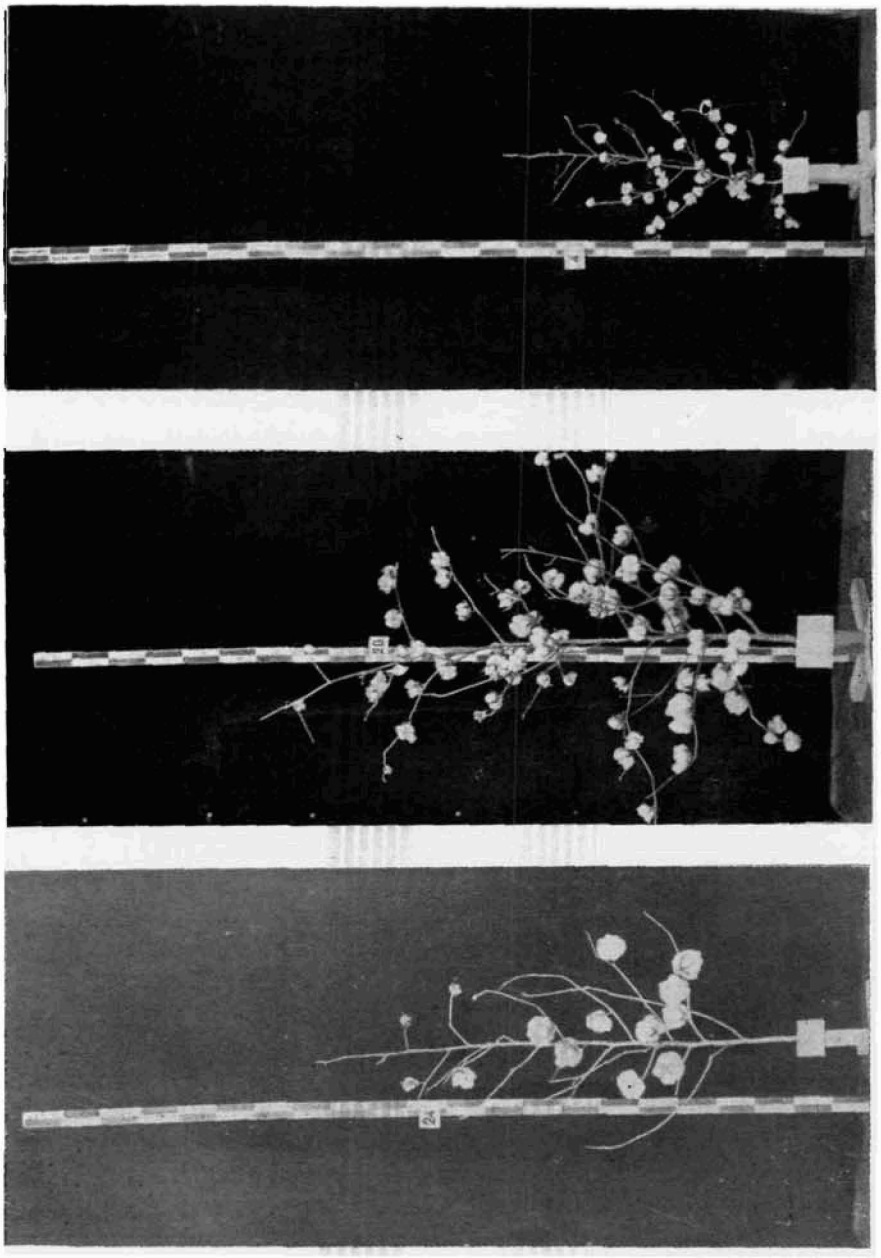
Thus, a great number of facts speaks about introgressive hybridization widely seen in nature beginning from ephemers and up to arboreal strains. But up till now there wasn't any hypothesis explaining the biological mechanism of this phenomenon, which is extremely interesting in the theoretical and practical aspects. The occasional appearance of alien pollen on the plant stigma may be the starting point for intercrossing. This pollen either penetrates the germ cell or influences the zygote pouring its content with the main mass of maternal pollen. Evidently this process taking place in nature demands a rather long period of time.

Methods and Results

Experimental modelling of introgression by L.I. Gurevich and us was begun in 1946 (D. V. Ter-Avanesian, 1957). The methods of experiments was worked out in the following way. The marked cotton plants were divided into groups, each included 15 flowers. Emasculated flowers were pollinated by 20 pollen grains of paternal (alien) pollen and half an hour later the pollen from the maternal form plant was put on the same stigma in abundance. Consequently if the first group was pollinated in half an hour, and the last, the 8th, 4 hours later. The greatest number of plants with the features of alien paternal form was in that variant where the 'maternal pollination' was made 3 hours later.

In the second variant of pollination, a lot of pollen from the flowers of the same plant was put on the stigma of emasculated flower and three hours later 20 pollen grains of alien species were put on the same stigma. In this case we imitated the process of pollen appearance on the stigma in natural conditions.

Plant emasculation made by us is explained by the necessity of fixation of the beginning of pollen germination from their own flower and from the second paternal form (alien pollination). Further we chose the first variant of pollination as there the effect of artificial introgression was discovered quicker. After thorough examination of the plants obtained from such pollination, there was found a number of plants diverging from the maternal form according to morphological features. In F_2 (sometimes in F_3) features, marked in the first year, are expressed more obviously and there can be made selection according to



1 2 3

FIG. 1. ♀ C. 460 (*G. hirsutum* L.); FIG. 2. F_5 (C-460 × C-460, after 3 hours + 20 p.g.) FIG. 3. ♂ A-833 (*G. herbaceum* L.).

new economical and morphological features. Beginning with F_3–F_4, the plants become uniform for these features and during the next years they present genetically uniform lines. The appearance of paternal additional pollination features reminds of the shot film on which the picture of the thing gradually becomes visible in the developer. The artificial selection consolidates new features. Evidently, in natural conditions this process goes in another way, but it takes far more time. We shall give several examples of obtaining peculiar hybrids with features of different chromosome cotton species.

The pollen of the diploid species *Gossypium herbaceum* or *G. arboreum* placed on the stigma of emasculated flower of *G. hirsutum* gives in the progeny maternal plants, *G. hirsutum*, with the changed leaf lobule, which became either rounded or stretched. The fibre quality changes as well. Under the influence of Old World species it becomes considerably coarser and shorter like in parental species. The acquired features remain in the following generations.

Analogous picture of hereditary changes was obtained after the pollination by the *Hibiscus cannabinus* (*ambary*) pollen. The influence of alien pollen was expressed in the strong change (Table 1) of morphological and economical features of the variety C-1472 (*G. hirsutum*). As seen from the data significant changes took place in the features of variety C-1472; besides this morphological features were greatly changed. The average height of the variety C-1472 doesn't exceed 80–90 cm, but the majority of experimental plants had shortened sympodial branches, their height reached 120–130 cm. and resembled *ambary* in its structure.

TABLE 1

The influence of H. cannabinus *pollen on the properties of cotton variety C*-1472 (G. hirsutum) *in* F_3 *and* F_6

Parental forms and their hybrids	Yield from 1 plant (g)	The height height of the plant s(sm)	Early ripening (days)	The boll weight	Fibre length (mm)	Fibre output %
C-1472 (G. hirsutum)	89,3	84	127	6,5	34,5	38,3
H. cannabinuss	—	145	—	—	—	—
C-1472 × H. cannab. F_3	47,9	128	127	3,6	25,1	34,7
— ,, —F_6	45,6	130	122	3,8	24,7	32,1

G. Kamalova studied histochemically the pollen germination of the representatives of Malvaceae family on the cotton stigma and she obtained following results. When pollinating the cotton flowers by the pollen of *Cienfuegosia*

(phglogenetically close genus), the pollen tubes reached the base of the style, and when pollinating by pollen of *Hibiscus* species from the section Trionum (*H. grandiflorus, H. trionum, H. militaris*) the pollen tubes grew all together. It is interesting, that according to the data of the same author, the pollen of the distant (in systematic respect) species (*Althaea brousonetiifolia, A. officinalis, Lavatera thuringiocea*) germinates well on the cotton stigma. The pollen tubes of the above-mentioned species even penetrate into the ovule. Putting the pollen of *Althea rosea* on the fast ripening variety C-1472 in the progeny we obtained plants with monopodial branches and with thin pendant sympodial branches of light anthocyanin colour with the changed form of the boll on them. Seed progeny of such plants were stable in transferrence of the acquired features during ten generations.

Further experiments with the participation of wild diploid species conducted by L. I. Gurevich, gave the following results. (1) Maternal fast-ripening cotton variety C-4727 (hairy seeds) was pollinated according to the above described method adding the pollen of *G. davidsonii*. In F_1, all the plants were of the maternal type (C-4727); in F_2, there appeared single plants the seeds of which didn't contain germs; F_3, there were plants with 8–10 monopodial branches. The height of the first sympodial branch is on the 12–13 node whereas in C. 4727 it is on the 5–6 node. 2) Maternal variety was the same C-4727. *G. armorianum* was the additional pollinator. In F_2, plants of shrub type with 8–12 monopodial branches, photoperiodical were obtained. (3) The same maternal type. The weight of seed cotton in the boll 6g. The additional pollinator *G. hopi* with small bolls from 2·5 to 4 g. In F_2 there appear plants with small bolls, 2·5–5 g. Seeds with all transitions—from bare up to hairy. There can be met single plants with green fibre and typical maternal plants with anthocyanin spot at the base of the flower petals. When backcrossing the experimental plants the new features remain in the following generations. The pollination by *G. trilobum* pollen gives in progeny changes in the colour of leaves, in the fuzz of seeds (bareseed forms appeared), part of seedlings lacked chlorophyll, with lethal gene.

L. I. Gurevich used the above method and pollinated susceptible to wilt variety C-4727 by pollen of wild highly resistant to wilt subspecies *mexicanum* var., *nervosum*. It is necessary to say that the indicated subspecies easily crosses with *G. hirsutum*, but the obtained hybrid in ordinary crossing needs repeated backcrossing and numerous selection to give this hybrid progeny features of cultivated cotton. When pollinating by 20 pollen grains (according to the above mentioned method) of *mexicanum* pollen, in the progeny of such plants there were singled out lines whch do not differ from maternal variety C-4727 according to phenotype, but with high resistance to wilt acquired from ssp. *mexicanum*. At present these lines, as two varieties "Express I" and "Express 2" occupy large areas in the northern regions of Uzbekistan.

There can be given numerous examples accumulated during the last three decades of years.

Discussion

It can be admitted that introgression in natural conditions, apparently, must occur in the following way: with the help of wind or insects, a small number of pollen grains of alien species (genus, family and so on) appears on the flower stigma. Two possibilities can be assumed in natural hybridization. Without barrier of sexual incompatibility, there can be admitted the penetration into the ovary of less related pollen, which occasionally got on the stigma in the presence of the own pollen and this is rather difficult becaue of the slow growth of the single pollen grains (Ter-Avanesian., 1949). As the result of the barrier of sexual incompatibility, the pollen tubes do not germinate into the ovule and do not fuse with the egg cell, but leave some physiologo-biochemical "trace". During some (short or long) period of time there occurs the repeated appearance (backcross) of alien pollen on the flower stigma of the same flower (if it is a perennial plant) or on the progeny of the plant, which was once affected by the pollen. It should be supposed that under the influence of alien pollen and in course ofinterrelation between the pollen tube content and the cytoplasm of the fertilized object there appears new hybrid between species which do not cross in the ordinary way.

The alien pollen, which is laid on in the additional pollination doesn't participate directly in the double fertilization but it brings metabolically its "materials" into the developing embryos.

In future, for the understanding of these phenomena, it will be necessary, apparently to study them using the biochemical methods on the level of molecular genetics as it can be assumed that some substances from these pollen tubes can penetrate into cytoplasm and affect the developing egg cell.

We expressed also the hypothesis about the influence of alien pollination as a kind of mutagen factor (D. V. Ter-Avanesian, 1973). But, if the ordinary physical and chemical mutagens are of occasional (not directed) character, the influence of alien pollen still gives in the progeny the variability of the same type, according to the features of paternal additional pollinator, which indicates a different action as compared to mutagen variability.

Thus, theoretical significance of the above work consists in the attempt to express the hypothesis about the participation of the alien pollen, explaining the effect of introgressive hybridization which takes place in the vegetative world. In the practical respect, the proposed method will allow to include into hybridization gene plasm of wild cotton species different in chromosomes and distant representatives of mallow family, bearing valuable features, which are lacking in the cultivated species.

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